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DETERMINATION OF CLETHODIM AND METABOLITES IN SOIL
METHOD: RM-26-S-1

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INTRODUCTION

This method describes the determination of clethodim and its metabolites (clethodim sulfoxide, clethodim sulfone, oxazole sulfoxide and oxazole sulfone) in soil. Briefly, this method involves the extraction of soil with methanol/water, partitioning into hexane and methylene chloride, derivatization with diazomethane, base wash and silica Sep-Pak cleanup; metabolite analysis is performed by HPLC at 254 nm with a C-1 column and clethodim analysis is performed with a C-18 column.

REAGENTS

BEST AVAILABLE COPY

Methanol - Pesticide grade

Water - Deionized

Hexane - Pesticide grade

Methylene Chloride - Pesticide grade

Hydrochloric Acid - Concentrated reagent grade

Sodium Chloride - Reagent

Sodium Sulfate - Reagent, anhydrous granular

Diazomethane (ether solution) - Prepared according to Aldrichimica Acta, 16, 3 (1983) or Aldrich Bulletin No. AL-113.

Acetone - Pesticide grade

Sodium hydroxide - 0.1 N solution

Silica Sep-Pak - Waters Associates

APPARATUS

Omni-Mixer with adapters for 1-pt. Mason jars.

Rotary vacuum evaporators with a water bath (maintain below 30° C).

High Performance Liquid Chromatograph

Hewlett-Packard 1090 (or equivalent) equipped with an autosampler and UV (254 nm) detector.

PROCEDURE

Weigh 20 grams of the soil sample into 1-pint Mason jars. For recovery purposes, a 20-gram untreated soil sample should be fortified with 100 µL of an acetone solution containing 20 µg each of clethodim sulfoxide, clethodim sulfone, oxazole sulfoxide and oxazole sulfone; another 20-gram untreated soil sample should be fortified with 100 µL of an acetone solution of clethodim (20 µg/mL). Add 150 mL of 80/20 methanol/water to the soil and mix the sample for 5 minutes on the Omni-Mixer. Vacuum filter the extract through #42 filter paper in a Buchner funnel into a 500-mL vacuum flask. Place the filter disk back into the Mason jar and gently mix again with 150 mL of 80/20 methanol/water (do not mix vigorously with the Omni-Mixer during this second extraction); vacuum filter the extract again, wash the filter with 50 mL of 80/20 methanol and combine the extracts and wash into a 1-liter separatory funnel. Add 300 mL water, 1 mL concentrated hydrochloric acid, and 10 g sodium chloride and extract the mixture by shaking with 100 mL hexane for 1 minute; drain the bottom aqueous layer into another container and transfer the hexane extract through sodium sulfate into a 500-mL roundbottom flask. Repeat the hexane extraction with another 100 mL of hexane, combine the hexane extracts and evaporate to dryness for clethodim analysis. Extract the aqueous layer with 3 x 100 mL methylene chloride, filter the extracts through sodium sulfate into a roundbottom flask and evaporate to dryness for Metabolite analysis.

Metabolites

Transfer the residue from the above methylene chloride extraction with 2 + 2 + 2 mL of acetone to a 10-mL Reactivial; add approximately 3 mg of silica gel to the vial. Quickly add 1 mL of diazomethane (ether solution) to the vial; cap the vial, shake and allow to sit for 15 minutes at room temperature with occasional shaking. Evaporate the solution under a stream of nitrogen to a volume of approximately 3 mL; decant the solution (care is taken to leave silica gel behind) into a 50-mL roundbottom flask. Rinse the vial with three 5-mL portions of acetone, decant the acetone into the roundbottom flask; evaporate (rotary) the solution to dryness.

Take up the residue in 25 mL of methylene chloride and transfer to a 125-mL sep funnel; rinse the roundbottom flask with several 5 mL portions of methylene chloride into the sep funnel. Extract the methylene chloride solution with 10 mL of 0.1 N sodium hydroxide solution followed with 10 mL of water; discard the aqueous portions, filter the organic layer through sodium sulfate into a 50-mL roundbottom flask and evaporate to dryness.

Take up the residue in 5 mL methylene chloride and transfer onto silica gel Sep-Pak (previously washed with 5 mL acetone, 10 mL methylene chloride); rinse the roundbottom flask with 2 mL methlyene chloride which is also transferred to the Sep-Pak. Elute the Sep-Pak with exactly 5 mL 10% acetone/methylene chloride (discard) and then elute the desired products into a 50-mL roundbottom flask with 5 + 5 mL of acetone. Evaporate the eluate to dryness and dissolve the residue in 1.0 mL of 30% acetonitrile/water for HPLC measurement.

Clethodim

Follow above METABOLITE procedure with residue from hexane extraction, but skip paragraph 2 of METABOLITE procedure. Follow above Sep-Pak procedure except skip elution with 10% acetone/methylene chloride. Take acetone eluent to dryness, reconstitute in 1 mL 30% acetonitrile/water, and split the sample into two autosampler vials for HPLC measurement.

Standards

Place 1 or 2 mL of 20 μg/mL acetone solution of clethodim (fortifying solution) in a 10-mL Reactivial and 1 or 2 mL of an acetone solution containing 20 μg each of clethodim sulfoxide, clethodim sulfone, oxazole sulfoxide and oxazole sulfone (fortifying solution) in another Reactivial; 4 mL acetone and 3 mg silica gel is added to each vial. Follow above METABOLITE Procedure at the diazomethane addition step, but, skip paragraph 2 of the METABOLITE procedure. For the metabolite standards, follow above Sep-Pak procedure and take the acetone eluent to dryness. For the clethodim standard, follow the Sep-Pak procedure except skip the elution with 10% acetone/methylene chloride and then take the acetone eluent to dryness. Reconstitute the products in an appropriate volume of 30% acetonitrile/water to produce the required linearity and shooting standards.

HPLC CONDITIONS

Metabolites (Conditions may vary to optimize resolution)

Column: Hypersil 3 μm, SAS, 150 x 4.6 mm or Hypersil 5μm, Butyl, 150 x 4.6 mm

Wavelength: 254 nm

Solvent: A - water; B - acetonitrile

Flow: 1.0 mL/min.

Gradient: t = 0 min	B = 20%
5	20
12	50
15	50
16	80
22	80

Stop Time: 23 min.

Post Time: 5 min.

Injection Volume: 20 μL

Clethodim (Conditions may vary to optimize resolution)

Column: Hypersil ODS, 3 μm, 250 x 2 mm
Wavelength: 254 nm
Solvent: A - water; B - acetonitrile
Flow: 0.3 mL/min.

Gradient: t = 0 min	B = 35%
5	35
15	60
25	60
26	100
36	100

Stop Time: 36 min.
Post Time: 10 min.
Injection Volume: 20 μL

MEASUREMENT

Transfer the solutions to be measured to auto sampler vials and load the sample tray as follows:

Metabolite Analysis: Metabolite standard, metabolite standard, sample, sample, etc., - a minimum of 5 metabolite standards should be interspersed throughout the run (standards are a concentration of 2 μg/mL). The linearity of the measurement should be verified at least weekly using metabolite standards at concentrations of 0.2, 0.5, 1.0, 2.0 and 5.0 μg/mL (as prepared above). The response factor (response equivalent to 1 μg/mL) should have a coefficient of variation ± 10% or less.

Clethodim Analysis: Clethodim standard, clethodim standard, sample, sample, etc., - a minimum of 5 clethodim standards should be interspersed throughout the run (standards are a concentration of 2 μg/mL). The linearity of the measurement should be verified at least weekly using clethodim standards at concentrations of 0.2, 0.5, 1.0, 2.0 and 5.0 μg/mL (as prepared above). The response factor (response equivalent to 1 μg/mL) should have a coefficient of variation ± 10% or less.

For the analysis of clethodim sulfoxide in the hexane (clethodim) fraction, half of the clethodim extracts are analyzed under the metabolite analysis conditions. Load the clethodim samples onto the metabolite analysis instrument in sequence following the metabolite samples: clethodim standard, metabolite standard, samples, clethodim standard, metabolite standard, samples, etc. - a minimum of 5 clethodim standards should be interspersed throughout the run (standards are at a concentration of 2 μg/mL).

The coefficient of variation for the reproducibility of reference standard peak heights/areas should be ± 10% or less.

Note: After the above methylation procedure, the compounds actually measured by HPLC are: the O-methylethers of clethodim, clethodim sulfoxide and clethodim sulfone; oxazole sulfoxide and oxazole sulfone. For simplicity, we do not refer to the derivative names in this method. Figures 1 and 2 show typical HPLC chromatograms of clethodim and metabolites (40 ng of each component on column).

CALCULATION

Metabolites:

$$\text{ppm} = \frac{\text{Peak Height (Sample)}}{\text{Average Peak Height (Standard)}} \times 2\,\mu g \times \frac{1}{20\,g}$$

Clethodim:

ppm = ppm clethodim (hexane fraction) + (0.96 x ppm clethodim sulfoxide (hexane fraction))

LIMIT OF DETECTION

The limit of detection is 0.01 ppm for clethodim and metabolites from 20 grams of soil.

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B. HO

Reviewed by:

BH:gt

cc: G. O. Breault
R & D Files
Residue Files

CALCULATIONS FOR CLETHODIM EQUIVALENTS

SO = clethodim sulfoxide
SEL = clethodim
SOSTD = clethodim sulfoxide standard
SELSTD = clethodim standard

using peak heights (or areas):

1. (SO [in SELSTD] / SO [in SOSTD]) x 2.0 = ug of SO in SELSTD
2. 2.0 - (ug of SO in SELSTD) = ug of SEL in SELSTD
3. (SEL [in sample]/SEL [in SELSTD]) x (ug of SEL in SELSTD) = ug SEL in sample
4. (SO [in sample]/SO [in SOSTD]) x 2.0 = ug SO in sample
5. ug SEL in sample + 1.04(ug SO in sample) = ug clethodim equivalents